

## **Amendments to the Claims:**

*This listing of claims will replace all prior versions, and listings, of claims in the application:*

1. (Currently Amended) A method for measuring the replication capacity of HBV, ~~e.g. HBV present in a biological sample, possibly in the presence of a pharmaceutical product, preferably an antiviral agent,~~ the method comprising:

~~(a) possibly extracting nucleic acids contained in the biological sample;~~

~~(b)~~(a) PCR amplifying HBV nucleic acids using at least two primer pairs selected so as to obtain at least two different amplified HBV genomic fragments which upon assembly represent a linear continuous DNA sequence transcriptable in pgRNA ;

~~(c)~~(b) cloning the fragments obtained under ~~(b)~~a into a vector under the control of an heterologous promoter, so producing a vector containing a linear continuous DNA sequence transcriptable in pgRNA under control of said promoter;

~~(d)~~(c) transfecting or transducing susceptible cells with the vector;

~~(e)~~(d) culturing the transfected or transduced cells in conditions allowing synthesis of HBV pgRNA from the cloned HBV DNA;

~~(f) possibly treating the cultured cells with the pharmaceutical product, in particular antiviral agent; and~~

~~(g)~~(e) determining the replication capacity of the HBV, ~~possibly incidence of the pharmaceutical product, preferably antiviral agent, on viral gene expression and/or viral replication.~~

2. (Original) The method according to claim 1, wherein the continuous DNA sequence comprises HBV nucleotides (from 5' to 3') :

- an about 1 genome unit starting in 5' from and including the nucleotide representing the +1 of transcription to the first nucleotide in 5' of the ATG of the pre-C gene, plus
  - a sub-genomic fragment starting from and including the A of the ATG of the pre-C gene and extending to and including the polyA attachment site,
- wherein the linear continuous DNA sequence does not comprise at its 5' end the ATG of the pre-C gene.

3. (Original) The method according to claim 2, wherein the continuous DNA sequence comprises from 5' to 3' nucleotides 1818 to 1813 and 1814 to 1960 of an HBV genomic sequence aligned with the sequence as set forth in GenBank AB048704.

4. (Original) The method according to claim 2, wherein the continuous DNA sequence comprises from 5' to 3' nucleotides 1816 to 1813 and 1814 to 2016 of an HBV genomic sequence aligned with the sequence as set forth in GenBank AB048704.

5. (Currently Amended) The method according to claim 2, wherein in step (b a), use is made of a first primer pair and of a second primer pair, comprising each a forward primer and a reverse primer designed to amplify respectively:

- a first fragment comprising a HBV DNA sequence comprising at its 5' end the nucleotide representing the +1 of transcription
- a second fragment comprising a HBV DNA sequence comprising at its 3' end the polyA attachment site

the 3' end of the first fragment and the 5' end of the second fragment being overlapping and preferably comprising a restriction site in the overlapping part.

6. (Original) The method according to claim 5, wherein the first primer pair comprises a forward primer A which is partially complementary to the 5' part of the pre-

C gene, including the nucleotide representing the +1 of transcription, but does not contain the ATG of the pre-C gene.

7. (Original) The method according to claim 6, wherein this forward primer A comprises a restriction site that is not present in the HBV genome.

8. (Original) The method according to claim 7, wherein this forward primer A comprises a restriction site chosen among *NotI*, *AscI*, *PacI*, *PmeI* and *Sse8387I*.

9. (Original) The method according to claim 8, wherein this forward primer A comprises a sequence as set forth in any one of SEQ ID NO: 1 to 12.

10. (Previously Presented) The method according to claim 5, wherein the first primer pair comprises a reverse primer B comprising a sequence as set forth in SEQ ID NO: 13 or 14, or a mixture of two reverse primers B comprising a sequence as set forth in SEQ ID NO: 13 and 14, respectively.

11. (Previously Presented) The method according to claim 5, wherein the second primer pair comprises a forward primer complementary to a region of HBV DNA which is in 5' with respect to that complementary with the reverse primer of the first primer pair.

12. (Original) The method according to claim 11, wherein the forward primer of the second primer pair and the reverse primer of the first primer pair are complementary to HBV genomic regions which overlap and comprise a unique natural restriction site.

13. (Original) The method according to claim 12, wherein the forward primer of the second primer pair and the reverse primer of the first primer pair comprise a *NcoI* site which is complementary to the unique natural *NcoI* site.

14. (Previously Presented) The method according to claim 11, wherein the forward primer comprises a sequence as set forth in SEQ ID NO: 15 or 16, or is a mixture of two primers comprising a sequence as set forth in SEQ ID NO: 15 and 16, respectively.

15. (Previously Presented) The method according to claim 5, wherein the second primer pair comprises a reverse primer D designed to be complementary to a region of the HBV genome which is in 3' of the polyA attachment site.

16. (Original) The method according to claim 15, wherein the reverse primer D comprises a sequence as set forth in SEQ ID NO: 17 or 18, or is a mixture of two primers D comprising a sequence as set forth in SEQ ID NO: 17 and 18, respectively.

17. (Currently Amended) The method according to claim 1, wherein use is made of primers complementary to HBV genomic regions that are well conserved among HBV.  
~~From here numbers should be modified according to modification.~~

18. (Currently Amended) The method according to claim 1, wherein amplification involves from 20 to 60, ~~in particular from 30 to 50, preferably about 40 cycles.~~

19. (Previously Presented) The method according to claim 1, wherein the amplified HBV fragments are cloned into a vector in a one-step cloning procedure.

20. (Previously Presented) The method according to claim 1, wherein the +1 of transcription of the heterologous promoter is fused to the +1 of transcription of the HBV fragment.

21. (Previously Presented) The method according to claim 1, wherein the promoter is chosen among the group consisting of CMV-IE promoter, human ubiquitine C gene promoter, the promoter of EF-1 $\alpha$  gene, the early and late promoters of the SV40 virus, the LTR promoter of the Rous sarcoma virus, cytoskeleton gene promoters.

22. (Original) The method according to claim 21, wherein the promoter is the desmin promoter or the actin promoter.

23. (Original) The method according to claim 22, wherein the promoter is the chicken  $\beta$ -actin promoter.

24. (Previously Presented) The method according to claim 22, wherein the  $\beta$ -actin promoter is associated to the CMV-IE enhancer.

25. (Currently Amended) The method according to claim 1, wherein in step (d<sub>c</sub>), the cells are eukaryotic cells, ~~preferably of hepatocyte origin, preferably cell lines such as hepatoma cells.~~

26. (Original) The method according to claim 25, wherein the cells are directly transfected with the vector.

27. (Original) The method according to claim 25, wherein the vector is transferred into baculovirus and then the cells are transduced with the baculovirus.

28. (Original) The method according to claim 25, wherein the vector is transferred into a cell line to produce a stable cell line constitutively expressing HBV.

29. (Cancelled).

30. (Cancelled).

31. (Withdrawn) Polynucleotide useful as primer for HBV amplification, comprising a sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

32. (Withdrawn) Primer pair for HBV amplification comprising a forward primer comprising (1) a sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 or SEQ ID NO: 12, and (2) a reverse primer comprising a sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 14 or a mixture of a reverse primer comprising a sequence as set forth in SEQ ID NO: 13 and a reverse primer comprising a sequence as set forth in SEQ ID NO: 14.

33. (Withdrawn) Primer pair for HBV amplification comprising (1) a forward primer comprising a sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 16, or a mixture of a forward primer comprising a sequence as set forth in SEQ ID NO: 15 and a forward primer comprising a sequence as set forth in SEQ ID NO: 16, and (2) a reverse primer comprising a sequence as set forth in SEQ ID NO: 17 or SEQ ID NO: 18 or a mixture of a reverse primer comprising a sequence as set forth in SEQ ID NO: 17 and a reverse primer comprising a sequence as set forth in SEQ ID NO: 18.

34. (Withdrawn) Kit for HBV amplification, comprising a primer pair according to claim 32 and a primer pair (1) a forward primer comprising a sequence as set forth in SEQ ID NO: 15 or SEQ ID NO: 16, or a mixture of a forward primer comprising a sequence as set forth in SEQ ID NO: 15 and a forward primer comprising a sequence as set forth in SEQ ID NO: 16, and (2) a reverse primer comprising a sequence as set forth

in SEQ ID NO: 17 or SEQ ID NO: 18 or a mixture of a reverse primer comprising a sequence as set forth in SEQ ID NO: 17 and a reverse primer comprising a sequence as set forth in SEQ ID NO: 18.

35. (Withdrawn) Vector comprising:

- a continuous HBV DNA as defined in claim 1, and
- a promoter modified in 5' by the presence of a restriction site in 5' of the +1 of transcription, with the +1 of transcription of linear continuous HBV DNA and of the promoter being fused, the promoter controlling the synthesis of a pgRNA from the continuous HBV DNA post-cell-transfection.

36. (Withdrawn) Baculovirus or cell line comprising the vector of claim 35.

37. (New) The method according to claim 1, comprising before step (a) a step which is extracting nucleic acids contained in the biological sample.

38. (New) The method according to claim 1, comprising a step of treating the cultured cells of step (d) with a pharmaceutical product or a molecule.

39. (New) The method according to claim 38, wherein the pharmaceutical product is an antiviral agent.

40. (New) The method according to claim 39, wherein the antiviral agent is lamivudine, adefovir dipivoxil, entecavir, emtricitabine, clevudine, telbivudine, tenofovir, beta-L-FD4C or any combinations thereof.

41. (New) The method ~~according to~~ claim 38, wherein a molecule is tested as a potential antiviral agent.

42. (New) The method according to claim 38, wherein step (e) comprises the determination of the incidence of the pharmaceutical product on viral gene expression and/or replication.

43. (New) The method according to claim 1, wherein the HBV is an HBV present in a biological sample.

44. (New) The method according to claim 43, wherein the identity of the HBV present in the sample is unknown.

45. (New) The method according to claim 43, wherein the continuous DNA sequence comprises HBV nucleotides (from 5' to 3') :

- an about 1 genome unit starting in 5' from and including the nucleotide representing the +1 of transcription to the first nucleotide in 5' of the ATG of the pre-C gene, plus

- a sub-genomic fragment starting from and including the A of the ATG of the pre-C gene and extending to and including the polyA attachment site,

wherein the linear continuous DNA sequence does not comprise at its 5' end the ATG of the pre-C gene.

46. (New) The method according to claim 45, wherein the continuous DNA sequence comprises from 5' to 3' nucleotides 1818 to 1813 and 1814 to 1960 of an HBV genomic sequence aligned with the sequence as set forth in GenBank AB048704.

47. (New) The method according to claim 45, wherein the continuous DNA sequence comprises from 5' to 3' nucleotides 1816 to 1813 and 1814 to 2016 of an HBV genomic sequence aligned with the sequence as set forth in GenBank AB048704.



48. (New) The method according to claim 45, wherein in step (a), use is made of a first primer pair and of a second primer pair, comprising each a forward primer and a reverse primer designed to amplify respectively:

- a first fragment comprising a HBV DNA sequence comprising at its 5' end the nucleotide representing the +1 of transcription
- a second fragment comprising a HBV DNA sequence comprising at its 3' end the polyA attachment site,  
the 3' end of the first fragment and the 5' end of the second fragment being overlapping.

49. (New) The method according to claim 48, wherein the first primer pair comprises a forward primer A which is partially complementary to the 5' part of the pre-C gene, including the nucleotide representing the +1 of transcription, but does not contain the ATG of the pre-C gene.

50. (New) The method according to claim 49, wherein this forward primer A comprises a restriction site that is not present in the HBV genome.

51. (New) The method according to claim 50, wherein this forward primer A comprises a restriction site chosen among NotI, AscI, PacI, PmeI and SSe8387I.

52. (New) The method according to claim 51, wherein this forward primer A comprises a sequence as set forth in any one of SEQ ID NO: 1 to 12.

53. (New) The method according to claim 48, wherein the first primer pair comprises a reverse primer B comprising a sequence as set forth in SEQ ID NO: 13 or 14, or a mixture of two reverse primers B comprising a sequence as set forth in SEQ ID NO: 13 and 14, respectively.

54. (New) The method according to claim 48, wherein the second primer pair comprises a forward primer complementary to a region of HBV DNA which is in 5' with respect to that complementary with the reverse primer of the first primer pair.

55. (New) The method according to claim 54, wherein the forward primer of the second primer pair and the reverse primer of the first primer pair are complementary to HBV genomic regions which overlap and comprise a unique natural restriction site.

56. (New) The method according to claim 55, wherein the forward primer of the second primer pair and the reverse primer of the first primer pair comprise a NcoI site which is complementary to the unique natural NcoI site.

57. (New) The method according to claim 54, wherein the forward primer comprises a sequence as set forth in SEQ ID NO: 15 or 16, or is a mixture of two primers comprising a sequence as set forth in SEQ ID NO: 15 and 16, respectively.

58. (New) The method according to claim 48, wherein the second primer pair comprises a reverse primer D designed to be complementary to a region of the HBV genome which is in 3' of the polyA attachment site.

59. (New) The method according to claim 58, wherein the reverse primer D comprises a sequence as set forth in SEQ ID NO: 17 or 18, or is a mixture of two primers D comprising a sequence as set forth in SEQ ID NO: 17 and 18, respectively.

60. (New) The method according to claim 43, wherein use is made of primers complementary to HBV genomic regions that are well conserved among HBV.

61. (New) The method according to claim 43, wherein amplification involves from 20 to 60 cycles.

62. (New) The method according to claim 43, wherein the amplified HBV fragments are cloned into a vector in a one-step cloning procedure.

63. (New) The method according to claim 43, wherein the +1 of transcription of the heterologous promoter is fused to the +1 of transcription of the HBV fragment.

64. (New) The method according to claim 43, wherein the promoter is chosen among the group consisting of CMV-IE promoter, human ubiquitine C gene promoter, the promoter of EF-1 $\alpha$  gene, the early and late promoters of the SV40 virus, the LTR promoter of the Rous sarcoma virus, cytoskeleton gene promoters.

65. (New) The method according to claim 64, wherein the promoter is the desmin promoter or the actin promoter.

66. (New) The method according to claim 65, wherein the promoter is the chicken  $\beta$ -actin promoter.

67. (New) The method according to claim 65, wherein the  $\beta$ -actin promoter is associated to the CMV-IE enhancer.

68. (New) The method according to claim 43, wherein in step (c), the cells are eukaryotic cells.

69. (New) The method according to claim 68, wherein the cells are directly transfected with the vector.

70. (New) The method according to claim 68, wherein the vector is transferred into baculovirus and then the cells are transduced with the baculovirus.

71. (New) The method according to claim 68, wherein the vector is transferred into a cell line to produce a stable cell line constitutively expressing HBV.

72. (New) The method according to claim 43, comprising a step of treating the cultured cells of step (d) with a pharmaceutical product or a molecule.

73. (New) The method according to claim 72, wherein the pharmaceutical product is an antiviral agent.

74. (New) The method according to claim 73, wherein the antiviral agent is lamivudine, adefovir dipivoxil, entecavir, emtricitabine, clevudine, telbivudine, tenofovir, beta-L-FD4C or any combinations thereof.

75. (New) The method according to claim 72, wherein a molecule is tested as a potential antiviral agent.

76. (New) The method according to claim 43, wherein the 3' end of the first fragment and the 5' end of the second fragment are overlapping and comprise a restriction site in the overlapping part.

77. (New) The method according to claim 61, wherein amplification involves from 30 to 50 cycles.

78. (New) The method according to claim 61, wherein amplification involves 40 cycles.